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Review

# Analytical aspects of microRNA in diagnostics: A review

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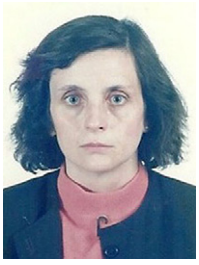
ABSTRACT

MicroRNAs (miRNA) are short (~22 nucleotides) non-coding RNA molecules that regulate gene expression at the post-transcriptional level. Their expression is specific to cells and tissues and is temporally regulated. miRNAs are known to be involved in developmental and physiological processes, and their dysregulation leads to development of diseases. Since their profiles reflect pathological processes, miRNAs have recently been proposed as being useful in diagnostics as biomarkers of the onset, prognosis and risk of diseases, as well as in the classification of different types of cancer. The establishment of miRNA profiles that are representative of diseases and the detection of different types and levels of miRNA in samples are therefore critical milestones in diagnostics. miRNAs can be detected in blood and body fluids as well as in tissues, thus making non-invasive collection of samples possible. For a method to be useful in diagnostics, it should be simple, inexpensive and highly sensitive. Here, we will review current methods of detecting miRNAs and indicate the advantages and disadvantages of each techniques. We will then summarize some of the clinical evidence for the potential application of miRNAs as biomarkers in diagnostics. We conclude providing some general perspectives on the use of miRNAs in clinical situations, including therapeutic applications.



**Mariangels de Planell Saguer** is a postdoctoral research scientist in the Motor Neuron Center at Columbia University in New York. She is characterizing the molecular functions of the SMN (Survival of Motor Neurons) protein complex and to define its role in the neuromuscular disease Spinal Muscular Atrophy (SMA). Her main research interest is aimed at understanding how the lack of the protein like SMN, with a housekeeping function in the building of the splicing machinery, can generate a diseases that mainly affects motor neurons. Understanding the origin of this vulnerability of the motor neurons to SMN deficiency is a critical step toward the development of successful therapeutic intervention for SMA. In 2008 she gained her PhD in Neuroscience (European degree) under the supervision of Prof. Zissimos Mourelatos and Dr María Celina Rodicio studying the function of IGHMBP2 protein and its role in the Spinal Muscular Atrophy with Respiratory Dystress (SMARD1) disease. Her PhD was awarded with Extraordinary Doctorate.



**María Celina Rodicio** is Professor in the Department of Cell Biology at the University of Santiago de Compostela (Spain). She received her PhD in Cell Biology from the University of Oviedo in 1977. Her research interests include development and organization of the nervous system and nervous system degeneration and regeneration, mainly using the lamprey as animal model. The current techniques in her laboratory are immunocytochemistry; anatomical pathway tracing with retrograde labels; electron microscopy, molecular cloning and *in situ* hybridization. Currently, she is investigating the possible role of neurotransmitters in spinal cord regeneration. She has published more than 60 scientific articles in peer-reviewed journals.

## 1. Introduction

While investigating the genes involved in chronic lymphocytic leukemia, Croce and co-workers discovered that mutations in a region of chromosome 13 were consistently associated with the disease, and that this region encodes two miRNAs [1]. This was the first evidence for the involvement of miRNAs in disease. miRNAs are ~22 nucleotide-long noncoding RNA molecules that regulate the expression of target genes at the post-transcriptional level by translational repression or degradation of messenger RNAs (mRNAs) [2–4]. An overview of miRNA biogenesis and function is shown in Fig. 1.

*Abbreviations:* AMI, acute myocardial infarction; FFPE, formalin fixed paraffin embedded; fM, femtomolar; ISH, *in situ* hybridization; LNA, locked nucleic acid; mRNA, messenger RNA; miRNA, microRNA; PNA, peptide nucleic acid; qRT-PCR, quantitative real-time polymerase chain reaction; Rluc, Renilla luciferase; SERS, surface-enhanced Raman spectroscopy; SPR, surface plasmon resonance; SPRI, SPR imaging.

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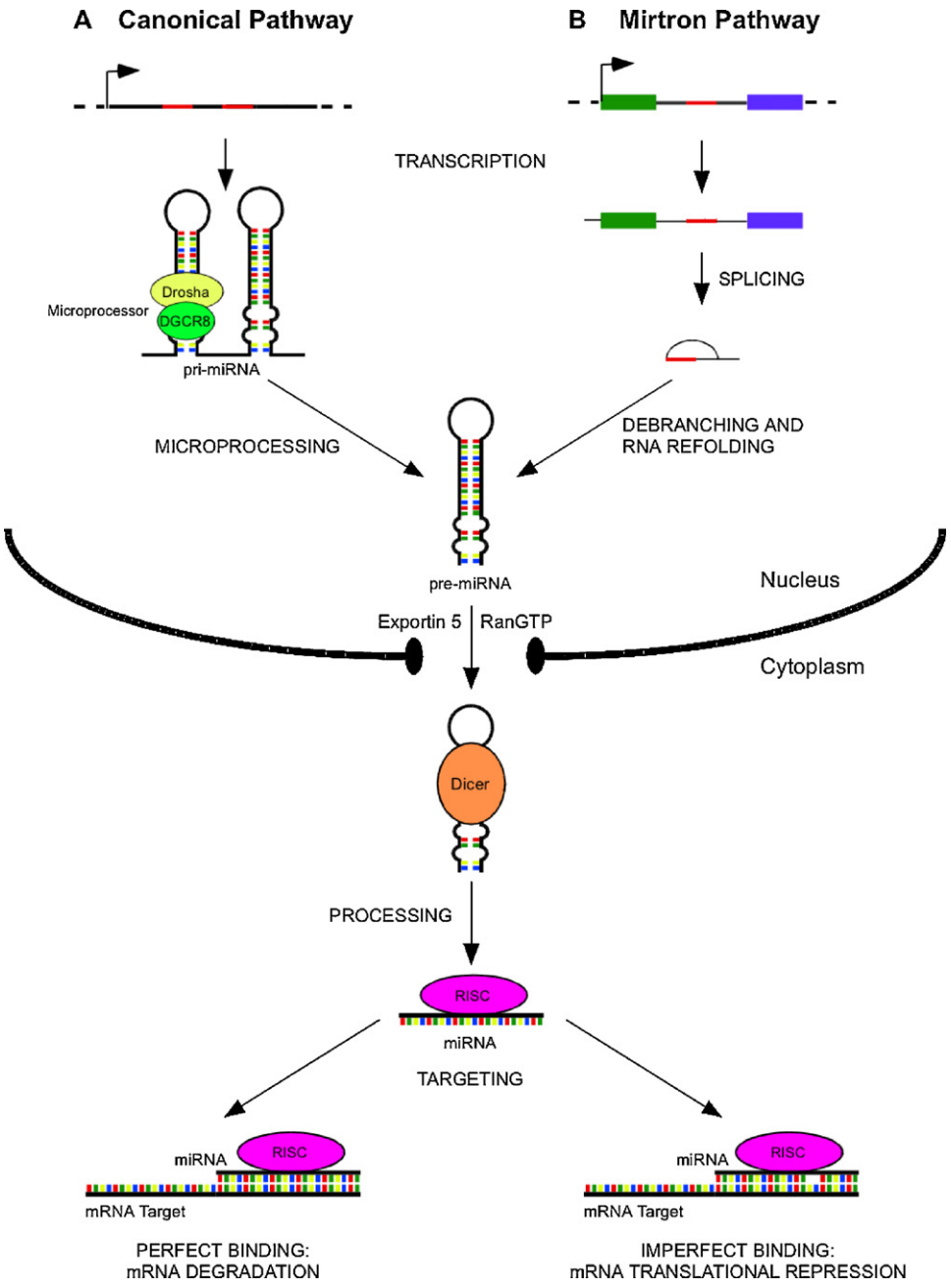


**Fig. 1.** Overview of miRNA biogenesis and function. Pri-miRNAs transcripts are processed in the nucleus to pre-miRNAs that are subsequently exported to the cytoplasm and processed to miRNAs that loaded into the RISC complexes. miRNAs bind to complementary sequences of their target mRNAs repressing their translation or inducing their degradation.

All human chromosomes, except the Y chromosome, have miRNA genes [5]. miRNAs are usually transcribed by RNA polymerase II into long primary transcripts with hairpin structures, known as pri-miRNAs, which are processed by the microprocessor complex in the nucleus, to form a harpin-like precursor miRNA (of about 70 nucleotides long) known as pre-miRNA. In addition, some pre-miRNAs can be produced by splicing and debranching of short introns known as mirtrons. Pre-mRNAs are exported to the cytoplasm by Exportin-5 and RanGTP where they are processed by the RNAse III endonuclease Dicer into short miRNA duplexes. One strand is the mature miRNA and the other, depicted with a star symbol (miRNA*), is degraded. The mature miRNA binds to an Argonaute protein to form the core component of miRNA microribonucleoprotein effector complexes. miRNAs bind to complementary sequences, typically in the 3′ untranslated regions of their target mRNAs. If miRNAs bind perfectly or almost perfectly to the miRNAs, they induce degradation of mRNA. If the binding is imperfect, the miRNA represses translation of the mRNA (review in [4]). Biogenesis of the microRNA can be regulated at a transcriptional level by regulatory factors, at a post-transcriptional level or at turnover of miRNAs (review in [6]).

It has been estimated that the human genome contains hundreds of miRNAs [7], which are thought to regulate thousands of protein-coding genes [8]. The miRNAs have been suggested to be involved in several biological process, such as developmental timing, proliferation, differentiation, metabolism, maintenance of cell and tissue identity, aging and cell death (for reviews, see [9,10]). Human miRNA sequences are available at the miRBase Sequence